Aldose Reductase Gene Polymorphisms and Diabetic Retinopathy Susceptibility

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OBJECTIVE — Aldose reductase (ALR) is involved in diabetic microvascular damage via the polyol pathway. A recent meta-analysis found genetic variation in the ALR gene (*AKR1B1*) to be significantly associated with diabetic retinopathy (DR). We investigated the genetic association of *AKR1B1* with DR.

RESEARCH DESIGN AND METHODS — The study enrolled 909 individuals with diabetes. Participants were genotyped for an *AKR1B1* (CA)n microsatellite and 14 tag single nucleotide polymorphisms, and ophthalmological assessment was performed.

RESULTS — A total of 514 individuals were found to have DR. rs9640883 was significantly associated with DR ($P = 0.0005$). However, *AKR1B1* variation was not independently associated with DR development after adjusting for relevant clinical parameters. rs9640883 was associated with duration of diabetes ($P = 0.002$).

CONCLUSION — Many previous reports have failed to account for known risk factors for DR. The commonly reported association of *AKR1B1* with DR may be due to an association of the gene with younger age at onset of diabetes.

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The polyol pathway is involved in microvascular damage, a hallmark of diabetic retinopathy (DR). Aldose reductase (ALR) is the first and rate-limiting enzyme in the polyol pathway, and pathogenic vascular and hemodynamic changes contributing to DR can subsequently occur as a result of sorbitol accumulation, oxidative damage, and protein kinase C activation (1–3). Over 160 candidate gene studies have been reported for DR, and their recent meta-analysis found genetic variation in the ALR gene (*AKR1B1*) to be the most significantly associated with DR (4). We aimed to determine whether genetic variation in the *AKR1B1* gene was associated with DR in a large cohort of Australian patients with type 1 or type 2 diabetes.

RESEARCH DESIGN AND METHODS

— After receiving approval from relevant Human Research Ethics Committees, participants diagnosed with type 1 diabetes ($n = 271$) or type 2 diabetes ($n = 638$) with a minimum 5-year duration were recruited from ophthalmology and endocrinology outpatient clinics of three tertiary hospitals in Adelaide, Australia. Retinopathy status for the worst eye was graded according to the Early Treatment Diabetic Retinopathy Study criteria (5). If either eye had clinically significant macular edema (CSME), irrespective of other DR gradings, the participant was also classified as having CSME. Blinding DR was defined as severe nonproliferative DR (NPDR), proliferative DR (PDR), or CSME.

Blood pressure, BMI, renal function tests, serum cholesterol, and A1C levels were obtained. Hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive medication at recruitment. Hypercholesterolemia was defined as total cholesterol of >5.5 mmol/l or use of lipid-lowering medication. Albuminuria was defined as urine albumin ≥30 mg/day.

The *AKR1B1* (CA)n microsatellite was PCR amplified using fluorescently labeled primers published by Ko et al. (6) in 883 individuals (263 with type 1 and 620 with type 2 diabetes) and genotyped by electrophoresis on an ABI PRISM 3100 (Applied Biosystems).

Using the tagger program implemented in Haploview 4.0, 14 tag single nucleotide polymorphisms (SNPs) across the *AKR1B1* gene, including the promoter region, were selected and genotyped in 909 individuals (271 with type 1 and 638 with type 2 diabetes) using iPLEX Gold chemistry on an autoflex mass spectrometer (Sequenom, San Diego, CA).

The χ^2 test for categorical and univariate binary logistic regression for continuous clinical covariates with DR were calculated in SPSS (version 15.0; SPSS, Chicago, IL). Allelic and genotypic associations of the (CA)n microsatellite were calculated using the χ^2 test and multivariate analysis with the binary logistic regression controlling for associated variables. Testing for association of all SNPs and haplotypes with DR was undertaken with the χ^2 test for univariate analysis and binary logistic for multivariate analysis in PLINK (version 1.06) (7) and also CLUMPHAP (8) when microsatellites were incorporated into haplotype analyses.

Bonferroni correction was applied to microsatellite and haplotype analyses. Multiple testing of individual SNPs was adjusted for using Nyholt's SNP spectral decomposition method (9), modified by Li and Ji (10), which estimated 10 independent tests.

RESULTS

— A total of 514 participants had DR, of which 311 had NPDR (95 with type 1 and 216 with type 2 diabetes), 188 had PDR (71 with type 1 and 117 with type 2 diabetes), and 150 had

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Table 1—*Associations of* AKR1B1 *tag SNPs with any diabetic retinopathy by type of diabetes*

	Type 1 diabetes*			Type 2 diabetes†		
SNP	Genotypic	Dominant	Recessive	Genotypic	Dominant	Recessive
rs17773344	0.9811	0.8839	0.9336	0.2914	0.1180	0.6861
rs9640883	0.3535	0.1694	0.9912	0.3103	0.1320	0.4681
rs12666691	0.3940	0.2484	0.6514	0.5757	0.3234	0.5455
rs782054	0.8583	0.5892	0.8072	0.3702	0.6078	0.1589
rs1708414	0.6800	0.4559	0.7998	0.2812	0.9537	0.1264
rs1791001	0.1219	0.5836	0.0705	0.7547	0.6625	0.4819
rs2259458	0.6934	0.4724	0.8101	0.4064	0.2774	0.2825
rs3896278	0.9337	0.7111	0.9123	0.9511	0.7548	0.9613
rs17188118	0.1979	0.1235	0.6781	0.3417	0.6348	0.2054
rs1424426	0.8324	0.6613	0.7891	0.9984	0.9569	0.9972
rs759853	0.8230	0.5850	0.6498	0.9903	0.9793	0.9024
rs1708403	0.6988	0.4594	0.8782	0.9803	0.9644	0.8423
rs1553976	0.2464	0.2269	0.4340	0.9419	0.9304	0.7698
rs4728326	0.6326	0.8303	0.3407	0.4734	0.3670	0.5761

Data are adjusted *P* values. *Adjusted for age, diabetes duration, hypertension, albuminuria, and high cholesterol. †Adjusted for sex, diabetes duration, hypertension, and A1C. *Note*: *P* values shown have not been corrected for multiple testing.

CSME (36 with type 1 and 114 with type 2 diabetes).

For the *AKR1B1* microsatellite, 13 alleles (120–144 bp) were present in our cohort. The 138 bp allele (24 repeats) was designated as the z allele. The z-10/z-8 genotype was significantly associated with blinding DR in type 1 diabetes ($P = 0.008$) and remained significant in the multivariate analysis ($P = 0.001$) and after correction for multiple testing ($P = 0.007$). However, only two and six participants with no DR and blinding DR respectively, carried this genotype, and the significance of this result is unclear. No association of the microsatellite with DR was detected in type 2 diabetes.

rs9640883 was significantly associated with DR in combined diabetes ($P = 0.0005$; odds ratio 1.62 [95% CI 1.24–2.13]), and type 2 diabetes ($P = 0.002$; 5.73 [4.26–7.69]) under the dominant model. This remained significant ($P < 0.005$) after correction for multiple testing. Haplotype analyses revealed no associations with DR. No tag SNP remained associated with DR after adjustment for associated covariates for type 1 (age, diabetes duration, hypertension, albuminuria, and hypercholesterolemia) and type 2 diabetes (sex, diabetes duration, hypertension, and A1C (Table 1). Each SNP was subsequently tested for association with each individual covariate in a univariate analysis. Of note, rs9640883 was associated with duration of diabetes under a dominant model ($P = 0.014$), particularly in the type 2 diabetes cohort ($P = 0.002$).

CONCLUSIONS — There have been numerous studies assessing polymorphisms of the *AKR1B1* gene and DR susceptibility, with (CA)n microsatellite and rs759853 most commonly studied. A recent meta-analysis found the z+2 allele in type 1 diabetes, and z−2 allele in any type of diabetes conferred protection from and risk for DR, respectively. The C allele of rs759853 conferred risk for DR in type 1 diabetes (4).

This study examined the (CA)n microsatellite and 14 tag SNPs. Although *AKR1B1* variation was associated with DR, once established risk factors including diabetes duration and A1C were considered, no association remained. This suggests that particular SNPs may be associated with the clinical covariates rather than having a direct association with DR.

We found the DR-associated SNP rs9640883 to also be associated with duration of diabetes. ALR reduces toxic aldehydes generated by reactive oxygen species to inactive alcohols. Decreased availability of the cofactor NADPH could induce or exacerbate intracellular oxidative stress (1). Chronic hyperglycemia and oxidative stress can result in permanent irreversible damage to pancreatic β-cells (11). Subsequent deterioration of β-cell function and increased disease severity results, with animal studies providing support for this hypothesis (12,13). Variation in ALR activity may affect the extent of oxidative stress, and genetic variation in *AKR1B1* may account for altered ALR activity. The association observed between rs9640883 and DR, and those previously reported for this gene, may reflect the effect this gene has on age of onset of diabetes and therefore on diabetes duration, in turn influencing DR risk (14,15).

The majority of previous studies examining the relationship between *AKR1B1* and DR have not undertaken multivariate analysis to consider known risk factors for DR. They may be influenced by the same confounding effect of duration of diabetes observed in this study.

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